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SEQUENCE LISTING

<110> TAKAKURA, Hikaru
MORISHITA, Mio
YAMAMOTO, Katsuhiko
MITTA, Masanori
ASADA, Kiyozo
TSUNASAWA, Susumu
KATO, Ikunoshin

<120> ULTRATHERMOSTABLE PROTEASE GENES

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<140> 09/841,553

<141> 2001-04-24

<150> 08/894,818

<151> 1997-08-29

<150> JP32385/1995

<151> 1995-12-12

<150> JP96/03253

<151> 1996-11-07

<160> 45

<170> PatentIn version 3.2

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<212> PRT

<213> Thermococcus celer

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Met Phe Gly Ser Tyr Gly Asp Arg Asp Arg Ala Val Lys Val Leu Arg
65 70 75 80

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Asp Thr Gly Tyr Phe Gly Asn Thr Arg Val Ser Gly Ile Lys Phe Ile
115 120 125

Gln Glu Asp Tyr Lys Val Gln Val Asp Asp Ala Thr Ser Val Ser Gln
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Ile Gly Ala Asp Thr Val Trp Asn Ser Leu Gly Tyr Asp Gly Ser Gly
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Val Val Val Ala Ile Val Asp Thr Gly Ile Asp Ala Asn His Pro Asp
165 170 175

Leu Lys Gly Lys Val Ile Gly Trp Tyr Asp Ala Val Asn Gly Arg Ser
180 185 190

Thr Pro Tyr Asp Asp Gln Gly His Gly Thr His Val Ala Gly Ile Val
195 200 205

Ala Gly Thr Gly Ser Val Asn Ser Gln Tyr Ile Gly Val Ala Pro Gly
210 215 220

Ala Lys Leu Val Gly Val Lys Val Leu Gly Ala Asp Gly Ser Gly Ser
225 230 235 240

Val Ser Thr Ile Ile Ala Gly Val Asp Trp Val Val Gln Asn Lys Asp
245 250 255

Lys Tyr Gly Ile Arg Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser
260 265 270

Ser Asp Gly Thr Asp Ser Leu Ser Gln Ala Val Asn Asn Ala Trp Asp
275 280 285

Ala Gly Ile Val Val Cys Val Ala Ala Gly Asn Ser Gly Pro Asn Thr
290 295 300

Tyr Thr Val Gly Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly
305 310 315 320

Ala Val Asp Ser Asn Asp Asn Ile Ala Ser Phe Ser Ser Arg Gly Pro
325 330 335

Thr Ala Asp Gly Arg Leu Lys Pro Glu Val Val Ala Pro Gly Val Asp
340 345 350

Ile Ile Ala Pro Arg Ala Ser Gly Thr Ser Met Gly Thr Pro Ile Asn
355 360 365

Asp Tyr Tyr Thr Lys Ala Ser Gly Thr Ser Met Ala Thr Pro His Val
370 375 380

Ser Gly Val Gly Ala Leu Ile Leu Gln Ala His Pro Ser Trp Thr Pro
385 390 395 400

Asp Lys Val Lys Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Ala Pro
405 410 415

Lys Glu Ile Ala Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Val Tyr
420 425 430

Lys Ala Ile Lys Tyr Asp Asp Tyr Ala Lys Leu Thr Phe Thr Gly Ser
435 440 445

Val Ala Asp Lys Gly Ser Ala Thr His Thr Phe Asp Val Ser Gly Ala
450 455 460

Thr Phe Val Thr Ala Thr Leu Tyr Trp Asp Thr Gly Ser Ser Asp Ile
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Asp Leu Tyr Leu Tyr Asp Pro Asn Gly Asn Glu Val Asp Tyr Ser Tyr
485 490 495

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Gly Thr Trp Thr Val Lys Val Val Ser Tyr Lys Gly Ala Ala Asn Tyr
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Gln Val Asp Val Val Ser Asp Gly Ser Leu Ser Gln Ser Gly Gly Gly
530 535 540

Asn Pro Asn Pro Asn Pro Asn Pro Asn Pro Thr Pro Thr Thr Asp Thr
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Gln Thr Phe Thr Gly Ser Val Asn Asp Tyr Trp Asp Thr Ser Asp Thr
565 570 575

Phe Thr Met Asn Val Asn Ser Gly Ala Thr Lys Ile Thr Gly Asp Leu
580 585 590

Thr Phe Asp Thr Ser Tyr Asn Asp Leu Asp Leu Tyr Leu Tyr Asp Pro
595 600 605

Asn Gly Asn Leu Val Asp Arg Ser Thr Ser Ser Asn Ser Tyr Glu His
610 615 620

Val Glu Tyr Ala Asn Pro Ala Pro Gly Thr Trp Thr Phe Leu Val Tyr
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<213> Thermococcus celer

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 <222> (428)..(428)
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35 40 45

Ile Gly Trp Val Asp Phe Val Asn Gly Arg Ser Tyr Pro Tyr Asp Asp
50 55 60

His Gly His Gly Thr His Val Ala Ser Ile Ala Ala Gly Thr Gly Ala
65 70 75 80

Ala Ser Asn Gly Lys Tyr Lys Gly Met Ala Pro Gly Ala Lys Leu Ala
85 90 95

Gly Ile Lys Val Leu Gly Ala Asp Gly Ser Gly Ser Ile Ser Thr Ile
100 105 110

Ile Lys Gly Val Glu Trp Ala Val Asp Asn Lys Asp Lys Tyr Gly Ile
115 120 125

Lys Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser Ser Asp Gly Thr
130 135 140

Asp Ala Leu Ser Gln Ala Val Asn Ala Ala Trp Asp Ala Gly Leu Val
145 150 155 160

Val Val Val Ala Ala Gly Asn Ser Gly Pro Asn Lys Tyr Thr Ile Gly
165 170 175

Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly Ala Val Asp Lys
180 185 190

Tyr Asp Val Ile Thr Ser Phe Ser Ser Arg Gly Pro Thr Ala Asp Gly
195 200 205

Arg Leu Lys Pro Glu Val Val Ala Pro Gly Asn Trp Ile Ile Ala Ala
210 215 220

Arg Ala Ser Gly Thr Ser Met Gly Gln Pro Ile Asn Asp Tyr Tyr Thr
225 230 235 240

Ala Ala Pro Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ile Ala
245 250 255

Ala Leu Leu Leu Gln Ala His Pro Ser Trp Thr Pro Asp Lys Val Lys
260 265 270

Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Lys Pro Asp Glu Ile Ala
275 280 285

Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Ala Tyr Lys Ala Ile Asn
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Tyr Asp Asn Tyr Ala Lys Leu Val Phe Thr Gly Tyr Val Ala Asn Lys
305 310 315 320

Gly Ser Gln Thr His Gln Phe Val Ile Ser Gly Ala Ser Phe Val Thr
325 330 335

Ala Thr Leu Tyr Trp Asp Asn Ala Asn Ser Asp Leu Asp Leu Tyr Leu
340 345 350

Tyr Asp Pro Asn Gly Asn Gln Val Asp Tyr Ser Tyr Thr Ala Tyr Tyr
355 360 365

Gly Phe Glu Lys Val Gly Tyr Tyr Asn Pro Thr Asp Gly Thr Trp Thr
370 375 380

Ile Lys Val Val Ser Tyr Ser Gly Ser Ala Asn Tyr Gln Val Asp Val
385 390 395 400

Val Ser Asp Gly Ser Leu Ser Gln Pro Gly Ser Ser Pro Ser Pro Gln
405 410 415

~~Pro Glu Pro Thr Val Asp Ala Lys Thr Phe Gln Xaa Ser Asp His Tyr~~
~~420 425 430~~

Tyr Tyr Asp Arg Ser Asp Thr Phe Thr Met Thr Val Asn Ser Gly Ala
435 440 445

Thr Lys Ile Thr Gly Asp Leu Val Phe Asp Thr Ser Tyr His Asp Leu
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Asp Leu Tyr Leu Tyr Asp Pro Asn Gln Lys Leu Val Asp Arg Ser Glu
465 470 475 480

Ser Pro Asn Ser Tyr Glu His Val Glu Tyr Leu Thr Pro Ala Pro Gly
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aaggctataa actacgataa ctatgcaaag ctagtggttca ctggatatgt tgccaacaaa 960
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Arg Asn Val Glu Lys Asn Tyr Gly Leu Leu Thr Pro Gly Leu Phe Arg
35 40 45

Lys Ile Gln Lys Leu Asn Pro Asn Glu Glu Ile Ser Thr Val Ile Val
50 55 60

Phe Glu Asn His Arg Glu Lys Glu Ile Ala Val Arg Val Leu Glu Leu
65 70 75 80

Met Gly Ala Lys Val Arg Tyr Val Tyr His Ile Ile Pro Ala Ile Ala
85 90 95

Ala Asp Leu Lys Val Arg Asp Leu Leu Val Ile Ser Gly Leu Thr Gly
100 105 110

Gly Lys Ala Lys Leu Ser Gly Val Arg Phe Ile Gln Glu Asp Tyr Lys
115 120 125

Val Thr Val Ser Ala Glu Leu Glu Gly Leu Asp Glu Ser Ala Ala Gln
130 135 140

Val Met Ala Thr Tyr Val Trp Asn Leu Gly Tyr Asp Gly Ser Gly Ile
145 150 155 160

Thr Ile Gly Ile Ile Asp Thr Gly Ile Asp Ala Ser His Pro Asp Leu
165 170 175

Gln Gly Lys Val Ile Gly Trp Val Asp Phe Val Asn Gly Arg Ser Tyr
180 185 190

Pro Tyr Asp Asp His Gly His Gly Thr His Val Ala Ser Ile Ala Ala
195 200 205

Gly Thr Gly Ala Ala Ser Asn Gly Lys Tyr Lys Gly Met Ala Pro Gly
210 215 220

Ala Lys Leu Ala Gly Ile Lys Val Leu Gly Ala Asp Gly Ser Gly Ser
225 230 235 240

Ile Ser Thr Ile Ile Lys Gly Val Glu Trp Ala Val Asp Asn Lys Asp
245 250 255

Lys Tyr Gly Ile Lys Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser
260 265 270

Ser Asp Gly Thr Asp Ser Leu Ser Gln Ala Val Asn Asn Ala Trp Asp
275 280 285

Ala Gly Ile Val Val Cys Val Ala Ala Gly Asn Ser Gly Pro Asn Thr
290 295 300

Tyr Thr Val Gly Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly
305 310 315 320

Ala Val Asp Ser Asn Asp Asn Ile Ala Ser Phe Ser Ser Arg Gly Pro

325

330

335

Thr Ala Asp Gly Arg Leu Lys Pro Glu Val Val Ala Pro Gly Val Asp
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Ile Ile Ala Pro Arg Ala Ser Gly Thr Ser Met Gly Thr Pro Ile Asn
355 360 365

Asp Tyr Tyr Thr Lys Ala Ser Gly Thr Ser Met Ala Thr Pro His Val
370 375 380

Ser Gly Val Gly Ala Leu Ile Leu Gln Ala His Pro Ser Trp Thr Pro
385 390 395 400

Asp Lys Val Lys Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Ala Pro
405 410 415

Lys Glu Ile Ala Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Val Tyr
420 425 430

Lys Ala Ile Lys Tyr Asp Asp Tyr Ala Lys Leu Thr Phe Thr Gly Ser
435 440 445

Val Ala Asp Lys Gly Ser Ala Thr His Thr Phe Asp Val Ser Gly Ala
450 455 460

Thr Phe Val Thr Ala Thr Leu Tyr Trp Asp Thr Gly Ser Ser Asp Ile
465 470 475 480

Asp Leu Tyr Leu Tyr Asp Pro Asn Gly Asn Glu Val Asp Tyr Ser Tyr
485 490 495

Thr Ala Tyr Tyr Gly Phe Glu Lys Val Gly Tyr Tyr Asn Pro Thr Ala
500 505 510

Gly Thr Trp Thr Val Lys Val Val Ser Tyr Lys Gly Ala Ala Asn Tyr
515 520 525

Gln Val Asp Val Val Ser Asp Gly Ser Leu Ser Gln Ser Gly Gly Gly
530 535 540

Asn Pro Asn Pro Asn Pro Asn Pro Asn Pro Thr Pro Thr Thr Asp Thr
545 550 555 560

Gln Thr Phe Thr Gly Ser Val Asn Asp Tyr Trp Asp Thr Ser Asp Thr
565 570 575

Phe Thr Met Asn Val Asn Ser Gly Ala Thr Lys Ile Thr Gly Asp Leu
580 585 590

Thr Phe Asp Thr Ser Tyr Asn Asp Leu Asp Leu Tyr Leu Tyr Asp Pro
595 600 605

Asn Gly Asn Leu Val Asp Arg Ser Thr Ser Ser Asn Ser Tyr Glu His
610 615 620

Val Glu Tyr Ala Asn Pro Ala Pro Gly Thr Trp Thr Phe Leu Val Tyr
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Ala Tyr Ser Thr Tyr Gly Trp Ala Asp Tyr Gln Leu Lys Ala Val Val
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Tyr Tyr Gly

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 <212> DNA
 <213> *Pyrococcus furiosus*

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actggcaatg	gttatgacat	tgcatatgtg	gatactgacc	ttgactacga	cttcaccgac	1080
gaagttccac	ttggccagta	caacgttact	tatgatgttg	ctgttttttag	ctactactac	1140
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Lys Glu Val Ser Gln Ala Ala Leu Asn Ala Ile Met Lys Gly Gln Pro
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Lys Thr Glu Leu Glu Lys Leu Gly Ala Glu Ile Leu Asp Glu Asn Arg
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Val Leu Asn Met Leu Leu Val Lys Ile Lys Pro Glu Lys Val Lys Glu
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Val Leu Asp Thr Gly Val Asp Pro Asn His Pro Phe Leu Ser Ile Thr
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Glu Ser Thr Gly Leu Met Glu Tyr Val Val Lys Thr Val Tyr Val Ser
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Ile Lys Pro Asn Val Val Ala Pro Gly Tyr Gly Ile Tyr Ser Ser Leu
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Pro Met Trp Ile Gly Gly Ala Asp Phe Met Ser Gly Thr Ser Met Ala
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Thr Pro His Val Ser Gly Val Val Ala Leu Leu Ile Ser Gly Ala Lys
595 600 605

Ala Glu Gly Ile Tyr Tyr Asn Pro Asp Ile Ile Lys Lys Val Leu Glu
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Ser Gly Ala Thr Trp Leu Glu Gly Asp Pro Tyr Thr Gly Gln Lys Tyr
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Thr Glu Leu Asp Gln Gly His Gly Leu Val Asn Val Thr Lys Ser Trp
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675 680 685

Val Ile Arg Gly Leu Tyr Ala Arg Asn Ser Ile Pro Asp Ile Val Glu
690 695 700

Trp His Ile Lys Tyr Val Gly Asp Thr Glu Tyr Arg Thr Phe Glu Ile
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Tyr Ala Thr Glu Pro Trp Ile Lys Pro Phe Val Ser Gly Ser Val Ile
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Leu Glu Asn Asn Thr Glu Phe Val Leu Arg Val Lys Tyr Asp Val Glu

740

745

750

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Glu Lys Phe Thr Pro Glu Asn Asn Tyr Thr Leu Thr Trp Tyr Asp Ile
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Asn Gly Pro Glu Met Val Thr His His Phe Phe Thr Val Pro Glu Gly
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Lys Phe Ala Ser Pro Val Thr 1280	Val Thr Val Thr 1285	Tyr Thr Ile Tyr 1290
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Glu Leu Ser Glu Gly Asn Ile 1355	Ile Gln Tyr Leu 1360	Gly Asp Ile Arg 1365

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Val Ala Ala Gly Asn Ser Gly Pro Asn Thr Tyr Thr Val Gly Ser Pro
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 cgactccgtc aacggcaggt cgacccccta cgatgaccag ggacacggaa cccacgttgc 1260
 gggatatcgtt gccggaaccg ggagcgttaa ctcccagtac ataggcgtcg gccccggcgc 1320
 gaagctcgtc ggcgtcaagg ttctcgttgc cgacggttcg ggaagcgtct ccaccatcat 1380
 cgcggtgtgt gactggaacg tccagaacta ggacaagtac gggataaggg tcatcaacct 1440
 ctccctcggc tctcccaga gctc 1464

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<400> 18
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<400> 19
ttttatcgat caggcgtccc aggcgttg 28

<210> 20
<211> 22
<212> DNA
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<220>
<223> Synthetic

<400> 20
cattataggt aagagaggaa tg 22

<210> 21
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<220>
<223> Synthetic

<400> 21
gatccattcc tctcttacct ataatggtac 30

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<211> 19
<212> DNA
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<220>
<223> Synthetic

<400> 22
tagcagtaat tgacacggg 19

<210> 23
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<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 23
tagcagtaat tgacactgg 19

<210> 24
<211> 22
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic

<400> 24

ctgttccagc tacgtgagtt cc

22

<210> 25

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 25

ctgttccagc tacatgagtt cc

22

<210> 26

<211> 507

<212> DNA

<213> Pyrococcus furiosus

<400> 26

a cta gtc atc tca ggt tta aca ggg ggt aaa gct aag ctt tca ggt gtt 49
Leu Val Ile Ser Gly Leu Thr Gly Gly Lys Ala Lys Leu Ser Gly Val
1 5 10 15

agg ttt atc cag gaa gac tac aaa gtt aca gtt tca gca gaa tta gaa 97
Arg Phe Ile Gln Glu Asp Tyr Lys Val Thr Val Ser Ala Glu Leu Glu
20 25 30

gga ctg gat gag tct gca gct caa gtt atg gca act tac gtt tgg aac 145
Gly Leu Asp Glu Ser Ala Ala Gln Val Met Ala Thr Tyr Val Trp Asn
35 40 45

ttg gga tat gat ggt tct gga atc aca ata gga ata att gac act gga 193
Leu Gly Tyr Asp Gly Ser Gly Ile Thr Ile Gly Ile Ile Asp Thr Gly
50 55 60

att gac gct tct cat cca gat ctc caa gga aaa gta att ggg tgg gta 241
Ile Asp Ala Ser His Pro Asp Leu Gln Gly Lys Val Ile Gly Trp Val
65 70 75 80

gat ttt gtc aat ggt agg agt tat cca tac gat gac cat gga cat gga 289
Asp Phe Val Asn Gly Arg Ser Tyr Pro Tyr Asp Asp His Gly His Gly
85 90 95

act cat gta gct tca ata gca gct ggt act gga gca gca agt aat ggc 337
Thr His Val Ala Ser Ile Ala Ala Gly Thr Gly Ala Ala Ser Asn Gly
100 105 110

aag tac aag gga atg gct cca gga gct aag ctg gcg gga att aag gtt 385
Lys Tyr Lys Gly Met Ala Pro Gly Ala Lys Leu Ala Gly Ile Lys Val
115 120 125

cta ggt gcc gat ggt tct gga agc ata tct act ata att aag gga gtt 433
Leu Gly Ala Asp Gly Ser Gly Ser Ile Ser Thr Ile Ile Lys Gly Val

130	135	140	
gag tgg gcc gtt gat aac aaa gat aag tac gga att aag gtc att aat			481
Glu Trp Ala Val Asp Asn Lys Asp Lys Tyr Gly Ile Lys Val Ile Asn			
145	150	155	160
ctt tct ctt ggt tca agc cag agc tc			507
Leu Ser Leu Gly Ser Ser Gln Ser			
165			
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<213>	Artificial Sequence		
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<210>	28		
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<212>	DNA		
<213>	Artificial Sequence		
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tctccaagga aaagtaattg ggtgggtaga			30
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<212>	DNA		
<213>	Artificial Sequence		
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			Met
			1
aag ggg ctg aaa gct ctc ata tta gtg att tta gtt cta ggt ttg gta			106
Lys Gly Leu Lys Ala Leu Ile Leu Val Ile Leu Val Leu Gly Leu Val			

	5	10	15	
gta ggg agc gta gcg gca gct cca gag aag aaa gtt gtt caa gta aga				154
Val Gly Ser Val Ala Ala Ala Pro Glu Lys Lys Val Val Gln Val Arg				
	20	25	30	
aat gtt gag aag aac tat ggt ctg cta acg cca gga ctg ttc aga aaa				202
Asn Val Glu Lys Asn Tyr Gly Leu Leu Thr Pro Gly Leu Phe Arg Lys				
	35	40	45	
att ccc aaa ttg gat cct aac gag gga atc agc aca gta att gta ttt				250
Ile Pro Lys Leu Asp Pro Asn Glu Gly Ile Ser Thr Val Ile Val Phe				
	50	55	60	65
gtt aac cat agg gga aaa gaa att gca gta aga gtt ctt gag tta atg				298
Val Asn His Arg Gly Lys Glu Ile Ala Val Arg Val Leu Glu Leu Met				
	70	75	80	
ggt gcc caa gtt agg tat gtg tac cat att ata ccc cca ata gct gcc				346
Gly Ala Gln Val Arg Tyr Val Tyr His Ile Ile Pro Pro Ile Ala Ala				
	85	90	95	
gat ctt aag gtt aga gac tta cta gtc atc tca ggt tta aca ggg ggt				394
Asp Leu Lys Val Arg Asp Leu Leu Val Ile Ser Gly Leu Thr Gly Gly				
	100	105	110	
gaa act aag ctt tca ggt gtt agg t				419
Glu Thr Lys Leu Ser Gly Val Arg				
	115	120		

<210> 31
 <211> 180
 <212> DNA
 <213> Pyrococcus furiosus

<400> 31	
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aagcttcata ccaaaaatca gtgagtatga aaatttagta attctaagga cgttttcaaa	120
ggcgtttgga cttgctggaa ttagatgtgg atatatgata gcaaataaaa agattataga	180

<210> 32
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 32	
agagggatcc atgaaggggc tgaaagct	28

<210> 33
 <211> 30

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 33
agaggcatgc gctctagact ctgggagagt

30

<210> 34
<211> 1962
<212> DNA
<213> *Pyrococcus furiosus*

<400> 34
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gtagcggcag ctccagagaa gaaagttgaa caagtaagaa atgttgagaa gaactatggt 120
ctgctaacgc caggactggt cagaaaaatt caaaaattga atcctaacga ggaaatcagc 180
acagtaattg tatttgaaaa ccatagggaa aaagaaattg cagtaagagt tcttgagtta 240
atgggtgcaa aagttaggta tgtgtacat attatacccg caatagctgc cgatcttaag 300
gtagagact tactagtcac ctcagggtta acagggggta aagctaagct ttcagggtgtt 360
aggtttatcc aggaagacta caaagttaca gtttcagcag aattagaagg actggatgag 420
tctgcagctc aagttatggc aacttacgtt tggaacttgg gatatgatgg ttctggaatc 480
acaataggaa taattgacac tggaattgac gcttctcatc cagatctcca aggaaaagta 540
attgggtggg tagattttgt caatggtagg agttatccat acgatgacca tggacatgga 600
actcatgtag cttcaatagc agctgggtact ggagcagcaa gtaatggcaa gtacaaggga 660
atggctccag gagctaagct ggcgggaatt aaggttctag gtgccgatgg ttctggaagc 720
atatctacta taattaaggg agttgagtgg gccgttgata acaaagataa gtacggaatt 780
aaggtcatta atctttctct tgggtcaagc cagagctcag atggtactga cgctctaagt 840
caggctgtta atgcagcgtg ggatgctgga ttagttgttg tggttgccgc tggaaacagt 900
ggacctaaaca agtatacaat cggttctcca gcagctgcaa gcaaagttat tacagttgga 960
gccgttgaca agtatgatgt tataacaagc ttctcaagca gagggccaac tgcagacggc 1020
aggcttaagc ctgaggttgt tgctccagga aactggataa ttgctgccag agcaagtgga 1080
actagcatgg gtcaaccaat taatgactat tacacagcag ctctggggac atcaatggca 1140
actctcacg tagctggtat tgcagccctc ttgctccaag cacacccgag ctggactcca 1200
gacaaagtaa aaacagccct catagaaact gctgatatcg taaagccaga tgaaatagcc 1260

gatatagcct acggtgcagg tagggttaat gcatacaagg ctataaacta cgataactat 1320
gcaaagctag tggtcactgg atatgttgcc aacaaaggca gccaaactca ccagttcggtt 1380
attagcggag cttcgttcgt aactgccaca ttatactggg acaatgccaa tagcgacctt 1440
gatctttacc tctacgatcc caatggaaac caggttgact actcttacac cgcctactat 1500
ggattcgaaa aggttggtta ttacaaccca actgatggaa catggacaat taaggttgta 1560
agctacagcg gaagtgcaaa ctatcaagta gatgtggtta gtgatggttc cctttcacag 1620
cctggaagtt caccatctcc acaaccagaa ccaacagtag acgcaaagac gttccaagga 1680
tccgatcact actactatga caggagcgac acctttacaa tgaccgttaa ctctggggct 1740
acaaagatta ctggagacct agtgtttgac acaagctacc atgatcttga cctttacctc 1800
tacgatccta accagaagct tgtagataga tcggagagtc ccaacagcta cgaacacgta 1860
gaatacttaa cccccgcccc aggaacctgg tacttcctag tatatgccta ctacacttac 1920
ggttgggctt actacgagct gacggctaaa gtttattatg gc 1962

<210> 35
<211> 654
<212> PRT
<213> *Pyrococcus furiosus*

<400> 35

Met Lys Gly Leu Lys Ala Leu Ile Leu Val Ile Leu Val Leu Gly Leu
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Val Val Gly Ser Val Ala Ala Ala Pro Glu Lys Lys Val Glu Gln Val
20 25 30

Arg Asn Val Glu Lys Asn Tyr Gly Leu Leu Thr Pro Gly Leu Phe Arg
35 40 45

Lys Ile Gln Lys Leu Asn Pro Asn Glu Glu Ile Ser Thr Val Ile Val
50 55 60

Phe Glu Asn His Arg Glu Lys Glu Ile Ala Val Arg Val Leu Glu Leu
65 70 75 80

Met Gly Ala Lys Val Arg Tyr Val Tyr His Ile Ile Pro Ala Ile Ala
85 90 95

Ala Asp Leu Lys Val Arg Asp Leu Leu Val Ile Ser Gly Leu Thr Gly
100 105 110

Gly Lys Ala Lys Leu Ser Gly Val Arg Phe Ile Gln Glu Asp Tyr Lys
115 120 125

Val Thr Val Ser Ala Glu Leu Glu Gly Leu Asp Glu Ser Ala Ala Gln
130 135 140

Val Met Ala Thr Tyr Val Trp Asn Leu Gly Tyr Asp Gly Ser Gly Ile
145 150 155 160

Thr Ile Gly Ile Ile Asp Thr Gly Ile Asp Ala Ser His Pro Asp Leu
165 170 175

Gln Gly Lys Val Ile Gly Trp Val Asp Phe Val Asn Gly Arg Ser Tyr
180 185 190

Pro Tyr Asp Asp His Gly His Gly Thr His Val Ala Ser Ile Ala Ala
195 200 205

Gly Thr Gly Ala Ala Ser Asn Gly Lys Tyr Lys Gly Met Ala Pro Gly
210 215 220

Ala Lys Leu Ala Gly Ile Lys Val Leu Gly Ala Asp Gly Ser Gly Ser
225 230 235 240

Ile Ser Thr Ile Ile Lys Gly Val Glu Trp Ala Val Asp Asn Lys Asp
245 250 255

Lys Tyr Gly Ile Lys Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser
260 265 270

Ser Asp Gly Thr Asp Ala Leu Ser Gln Ala Val Asn Ala Ala Trp Asp
275 280 285

~~Ala Gly Leu Val Val Val Val Ala Ala Gly Asn Ser Gly Pro Asn Lys~~
~~290 295 300~~

Tyr Thr Ile Gly Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly
305 310 315 320

Ala Val Asp Lys Tyr Asp Val Ile Thr Ser Phe Ser Ser Arg Gly Pro
325 330 335

Thr Ala Asp Gly Arg Leu Lys Pro Glu Val Val Ala Pro Gly Asn Trp
340 345 350

Ile Ile Ala Ala Arg Ala Ser Gly Thr Ser Met Gly Gln Pro Ile Asn
355 360 365

Asp Tyr Tyr Thr Ala Ala Pro Gly Thr Ser Met Ala Thr Pro His Val
370 375 380

Ala Gly Ile Ala Ala Leu Leu Leu Gln Ala His Pro Ser Trp Thr Pro
385 390 395 400

Asp Lys Val Lys Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Lys Pro
405 410 415

Asp Glu Ile Ala Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Ala Tyr
420 425 430

Lys Ala Ile Asn Tyr Asp Asn Tyr Ala Lys Leu Val Phe Thr Gly Tyr
435 440 445

Val Ala Asn Lys Gly Ser Gln Thr His Gln Phe Val Ile Ser Gly Ala
450 455 460

Ser Phe Val Thr Ala Thr Leu Tyr Trp Asp Asn Ala Asn Ser Asp Leu
465 470 475 480

Asp Leu Tyr Leu Tyr Asp Pro Asn Gly Asn Gln Val Asp Tyr Ser Tyr
485 490 495

Thr Ala Tyr Tyr Gly Phe Glu Lys Val Gly Tyr Tyr Asn Pro Thr Asp
500 505 510

Gly Thr Trp Thr Ile Lys Val Val Ser Tyr Ser Gly Ser Ala Asn Tyr
515 520 525

Gln Val Asp Val Val Ser Asp Gly Ser Leu Ser Gln Pro Gly Ser Ser
530 535 540

Pro Ser Pro Gln Pro Glu Pro Thr Val Asp Ala Lys Thr Phe Gln Gly
545 550 555 560

Ser Asp His Tyr Tyr Tyr Asp Arg Ser Asp Thr Phe Thr Met Thr Val
565 570 575

Asn Ser Gly Ala Thr Lys Ile Thr Gly Asp Leu Val Phe Asp Thr Ser
580 585 590

Tyr His Asp Leu Asp Leu Tyr Leu Tyr Asp Pro Asn Gln Lys Leu Val
595 600 605

Asp Arg Ser Glu Ser Pro Asn Ser Tyr Glu His Val Glu Tyr Leu Thr
610 615 620

Pro Ala Pro Gly Thr Trp Tyr Phe Leu Val Tyr Ala Tyr Tyr Thr Tyr
625 630 635 640

Gly Trp Ala Tyr Tyr Glu Leu Thr Ala Lys Val Tyr Tyr Gly
645 650

<210> 36
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 36
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<210> 37
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<220>
<223> Synthetic

<400> 37
tgtactgctg gatccggcag 20

<210> 38
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<212> DNA
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<220>
<223> Synthetic

<400> 38
ggatccatca gatttttgag tgtagatcaa ccagtatgct gcatttgtaa ttgtgagata 60

atatctcccg cgggtaaggt 80

<210> 39
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 39
agaggcatgc gtatccatca gatttttgag

30

<210> 40
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<220>
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<400> 40
agtgaacgga tacttggaac

20

<210> 41
<211> 20
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<213> Artificial Sequence

<220>
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<400> 41
gttccaagta tccgttcact

20

<210> 42
<211> 12
<212> PRT
<213> Pyrococcus furiosus
<400> 42

Ala Glu Leu Glu Gly Leu Asp Glu Ser Ala Ala Gln
1 5 10

<210> 43
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Synthetic

<220>

<221> misc_feature
<222> (1)..(1)
<223> Leu is modified by a succinyl group.

<220>
<221> misc_feature
<222> (4)..(4)
<223> Tyr is modified by a 7-amino-4-methylcoumarin group.

<400> 43

Leu Leu Val Tyr
1

<210> 44
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Synthetic

<220>
<221> misc_feature
<222> (1)..(1)
<223> Ala is modified by a succinyl group.

<220>
<221> misc_feature
<222> (4)..(4)
<223> Phe is modified by a p-nitroaniline group.

<400> 44

Ala Ala Pro Phe
1

<210> 45
<211> 382
<212> PRT
<213> subtilisin

<400> 45

Met Arg Gly Lys Lys Val Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu
1 5 10 15

Ile Phe Thr Met Ala Phe Gly Ser Thr Ser Ser Ala Gln Ala Ala Gly
20 25 30

Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met
35 40 45

Ser Thr Met Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly
50 55 60

Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala Ala Ser Ala Thr
65 70 75 80

Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala
85 90 95

Tyr Val Glu Glu Asp His Val Ala His Ala Tyr Ala Gln Ser Val Pro
100 105 110

Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr
115 120 125

Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser
130 135 140

Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala Ser Met Val Pro Ser
145 150 155 160

Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His Gly Thr His Val Ala
165 170 175

Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala
180 185 190

Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu Gly Ala Asp Gly Ser
195 200 205

Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn
210 215 220

Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala
225 230 235 240

Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala Ser Gly Val Val Val
245 250 255

Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly Ser Ser Ser Thr Val
260 265 270

Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp

275

280

285

Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu Leu Asp
290 295 300

Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys
305 310 315 320

Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser Pro His Val Ala Gly
325 330 335

Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn Thr Gln
340 345 350

Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys Leu Gly Asp Ser Phe
355 360 365

Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala Ala Ala Gln
370 375 380